DETAILED ACTION

This application is a 371 of PCT/FR05/00070.

The amendment filed on December 16, 2011 has been entered.

Claims 1-6, 9-12, 16-17, 22-27, 32-36, 39-41, 46-47 and 50 are pending.

Response to Arguments

Applicant's amendment and arguments filed on December 16, 2011, have been fully considered and are deemed to be persuasive to overcome some of the objections/rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claim Objections

In view of the amendment of claims 10-11 and 40-41, the objection to claim10-11 and 40-41 has been **withdrawn.**

Claim Rejections - 35 USC § 112- 1st paragraph

In view of the amendment, the rejection of claims 1-6, 9-14, 16-17, 22-27, 30-36, and 39-50 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement, has been **withdrawn**.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Applicant's arguments, see pages 7-8 of the Remarks, filed December 16, 2011, with respect to the rejection(s) of claim(s) 1-2, 5-6, 9-14, 16, 22-24, 26-27, 30-32, 35-36, 39-46 and 48-50 under 35 U.S.C. 103(a) as being unpatentable over Cameron et al., Altaras et al., and Bermejo et al. have been fully considered and are persuasive. Therefore, the rejection has been withdrawn. However, upon further consideration, a new ground(s) of rejection is made in view of Harder et al.

Claims 1-2, 5-6, 9-12, 16, 22-24, 26-27, 32, 35-36, 39-41, 46, and 50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cameron et al., Altaras et al., Bermejo et al. and Harder et al.

Claims 1-2, 5-6, 9-12, 16, 22-24, 26-27, 32, 35-36, 39-41, 46, and 50 drawn to an *E. coli* comprising a deletion of its *tpiA*, *gloA,* and *ldhA* genes and expression of heterologous *adc*, *ctfAB* and *thl* genes from *C. acetobutylicum* and comprising evolved genes encoding enzymes that increases synthesis of 1,2-propanediol and a method of producing said *E. coli*, wherein the said *E. coli* is selected by applying increasing rates of dilution in such a way as to conserve in the growth medium only those *E. coli* strain that display a growth rate equal to or higher than the imposed rate of dilution.

Cameron et al. (US Patent No. 6,303,352 B1 – cited previously on form PTO-892) discloses a method of modifying *E. coli* to increase production of 1,2-propanediol by deleting its *tpiA* and/or *gloA* genes and over-expressing genes encoding enzymes that increases metabolism of pyruvate to acetate and/or pyruvate to acetyl-CoA and NADH, wherein said *E. coli* has improved 1,2-propanediol synthesis, and a method of producing said *E. coli* (Column 4, line 66 through Column 12, line 16). *E. coli* has an endogenous pyruvate dehydrogenase complex.

The difference between the reference of Cameron et al. and the instant invention is that the reference of Cameron et al. does not teach further "evolution" of the above *E. coli* by evolving genes involved in the biosynthesis pathway from DHAP to methylglyoxal and then to 1,2-propanediol, such as deletion of *ldhaA* and expression of

C. acetobutylicum gene encoding an enzyme that increases production of acetone and selecting said E. coli by the limitation recited in claim 1.

Altaras et al. (Biotechnol. Prog. 16:940-946 - form PTO-1449) discloses enhanced production of 1,2-propanediol by genetic engineering, comprising deletion of the *ldhaA* gene in *E. coli* (abstract and page 940). Altaras et al. teaches that elimination of the byproduct, lactate, increases production of 1,2-propanediol (abstract and page 940).

Bermejo et al. (Appl Environ Microbiol. 1998 Mar;64(3):1079-85 - form PTO-892) discloses expression of *C. acetobutylicum adc*, *ctfAB* and *thl* genes encoding an enzyme that increases production of acetone in *E. coli* in order to improve solvent production and an acetone producing *E. coli* may be useful hosts, which decreases the accumulation of detrimental acetate (page 936).

Selection of evolved microorganisms or *E. coli* by applying increasing rates of dilution in such a way as to conserve in the growth medium only those *E. coli* strain that display a growth rate equal to or higher than the imposed rate of dilution is a technique well known and established in the art, see Harder et al. (form PTO-892).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to further modify or evolve the recombinant *E. coli* of Cameron et al. by deleting it's *ldhA* genes and over-express a *C. acetobutylicum* gene encoding an enzyme that decreases accumulation of acetate or gldA and/or mgs and select the recombinant E. coli by the step recited in claim 1. One of ordinary skill in the art at the time the invention was made would have been motivated to do the above for

the purpose of eliminating production of the byproduct, lactate, and in order to increase production of 1,2-propanediol and decrease accumulation of acetate. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation for success sine Cameron et al. teaches deletion of the *tpiA* and *gloA* gene in E. coli to increase 1,2-propanediol production, Altaras et al. teaches mutant *E. coli*, comprising deletion of its *ldhA* gene and over-expression of gldA and mgs, having increased production of 1,2-propanediol, Bermejo et al. teaches expression of a *C. acetobutylicum* gene encoding an enzyme that increases production of acetone in *E. coli* in order to decrease accumulation of acetate and selection of mutant strains by the step recited in claim 1 was well known and practiced in the art.

Therefore, the above references render claims 1-2, 5-6, 9-12, 16, 22-24, 26-27, 32, 35-36, 39-41, 46, and 50 *prima facie* obvious.

In response to the previous Office Action, applicants have traversed the above rejection. Applicants should note that the rejection has been amended in light of the amendment of the claims.

Applicants argue that none of the references teach the evolution of E. coli as recited in the newly amended claim 1. The rejection has been amended. Selection of mutant/evolved strains by applying increasing rates of dilution in such a way as to conserve in the growth medium only those *E. coli* strain that display a growth rate equal to or higher than the imposed rate of dilution is a well known and practiced technique, see Harder et al.

Applicants argue that one of ordinary skill in the art would have no motivation to delete the ldhaA gene alone with an expectation that such a deletion would result in enhanced production of 1,2-propanediol because Altaras et al. teachs that the specific overexpression of certain genes in combination with the deletion of other genes may result in enhanced production of 1,2-propanediol. Examiner respectfully disagrees. The claims do not exclude modifying genes (either via inactivation or up-regulation) that are not recited in the claims. In order to further increase the 1,2-propanediol yield of the microorganism of Cameron et al., one having ordinary skill in the art would have been motivated to further modify the microorganism of Cameron et al. by adopting the various combinations of gene taught by Altaras et al.

Applicants also argue that there is no motivation as to why one of ordinary skill in the art would simultaneously delete *ldhaA* while at the same time increasing expression of a *C. acetobutylicum* gene. Examienr respectfully disagrees. One of ordinary skill in the art at the time the invention was made would have been motivated to do the above for the purpose of pushing the carbon flux towards 1,2-propanediol by eliminating production of the byproduct, lactate, and decrease accumulation of acetate.

Hence the rejection is maintained.

Conclusion

Allowable Subject Matter

Claims 3-4, 17 and 25 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Claims 1-2, 5-6, 9-12, 16, 22-24, 26-27, 32, 35-36, 39-41, 46, and 50 are rejected.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Yong Pak whose telephone number is 571-272-0935. The examiner can normally be reached 6:30 A.M. to 5:00 P.M. Monday through Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert Mondesi can be reached on 571-272-0956. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll free).

/Yong D Pak/
Primary Examiner, Art Unit 1652